

What is Claimed Is:

1. A high-throughput assay method for rapidly determining the proliferative status of a population of primitive hematopoietic cells, the method comprising the steps of:
 - 5 (a) providing a cell population comprising primitive hematopoietic cells;
 - (b) incubating the cell population in a cell growth medium comprising fetal bovine serum having a concentration of between 0% and 30% and methyl cellulose having a concentration of between about 0.4% and about 0.7%, and in an atmosphere having between about 3.5% oxygen and 7.5% oxygen;
 - 10 (c) contacting the cell population with a reagent capable of generating luminescence in the presence of ATP; and
 - 15 (d) detecting luminescence generated by the reagent contacting the cell population, the level of luminescence indicating the amount of ATP in the cell population, wherein the amount of ATP indicates the proliferative status of the primitive hematopoietic cells.
- 20 2. The method of Claim 1, wherein the concentration of fetal bovine serum is between about 0% and 10%.
3. The method of Claim 1, wherein the concentration of methyl cellulose is about 0.7%.
- 25 4. The method of Claim 1, wherein the concentration of oxygen in the atmosphere is about 5%.
- 30 5. The method of Claim 1, further comprising the step of contacting the primitive

hematopoietic cell population with at least one cytokine.

6. The method of Claim 5, further comprising the step of generating a cell population substantially enriched in hematopoietic stem cells.

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7. The method of Claim 5, further comprising the step of generating a cell population substantially enriched in at least one hematopoietic progenitor cell lineage.

- 10 8. The method of Claim 1, wherein the primitive hematopoietic cells are hematopoietic stem cells.

9. The method of Claim 1, wherein the primitive hematopoietic cells are hematopoietic progenitor cells.

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10. The method of Claim 1, wherein the population of primitive hematopoietic cells comprises hematopoietic stem cells and hematopoietic progenitor cells.

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11. The method of Claim 1, wherein the primitive hematopoietic cells are primary hematopoietic cells.

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12. The method of Claim 11, wherein the primary hematopoietic cells are isolated from an animal tissue selected from the group consisting of peripheral blood, bone marrow, umbilical cord blood, yolk sac, fetal liver and spleen.

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13. The method of Claim 12, wherein the animal tissue is obtained from a human.

14. The method of Claim 12, wherein the animal tissue is obtained from a mammal.

15. The method of Claim 14, wherein the mammal is selected from the group consisting of cow, sheep, pig, horse, goat, dog, cat, non-human primates, rodents, rabbit and hare.
- 5 16. The method of Claim 14, wherein the animal tissue is selected from bone marrow, yolk sac, fetal liver and spleen.
17. The method of Claim 13, wherein the animal tissue is human tissue further selected from the group consisting of peripheral blood, bone marrow, umbilical cord blood, fetal liver and spleen.
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18. The method of Claim 11, wherein the primary hematopoietic stem cells are isolated from peripheral blood.
- 15 19. The method of Claim 1, further comprising the step of selecting a differentially distinguishable subpopulation of primitive hematopoietic cells from the population of primitive hematopoietic cells, wherein the subpopulation of cells is defined by cell surface markers thereon.
- 20 20. The method of Claim 19, wherein the step of selecting a subpopulation of primitive hematopoietic cells comprises the steps of:
- (a) contacting the population of primitive hematopoietic cells with at least one cell surface marker indicator capable of selectively binding to a cell surface marker of a differentially distinguishable subpopulation of cells; and
- 25 (b) selectively isolating the at least one subpopulation of cells binding the at least one indicator.
21. The method of Claim 19, wherein the cell surface marker is selected from the group consisting of CD3, CD4, CD8, CD34, CD90 (Thy-1) antigen, CD117,
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CD38, CD56, CD61, CD41, glycophorin A and HLA-DR, AC133 defining a subset of CD34⁺ cells, CD19, and HLA-DR.

22. The method of Claim 19, wherein the cell surface marker is CD34⁺.
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23. The method of Claim 20, wherein the subpopulation of differentially distinguishable primitive cells is selectively isolated by magnetic bead separation.
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24. The method of Claim 20, wherein the subpopulation of differentially distinguishable primitive cells is selectively isolated by flow cytometry and cell sorting.
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25. The method of Claim 1, wherein the population of primitive hematopoietic cells comprises at least one stem cell lineage selected from the group consisting of colony-forming cell-blast (CFC-blast), high proliferative potential colony forming cell (HPP-CFC) colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM).
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26. The method of Claim 1, wherein the population of primitive hematopoietic cells comprises at least one hematopoietic progenitor cell lineage selected from the group consisting of granulocyte-macrophage colony-forming cell (GM-CFC), megakaryocyte colony-forming cell (CFC-mega), macrophage colony-forming cell (M-CFC), granulocyte colony forming cell (G-CFC),
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- burst-forming unit erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), colony-forming cell-basophil (CFC-Bas), colony-forming cell-eosinophil (CFC-Eo), colony-forming cell-megakaryocyte (CFC-Mega), B cell colony-forming cell (B-CFC) and T cell colony-forming cell (T-CFC).
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27. The method of Claim 1, wherein the reagent capable of generating

luminescence in the presence of ATP comprises luciferin and luciferase.

28. The method of Claim 5, wherein the at least one cytokine is selected from the group consisting of erythropoietin, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, thrombopoietin, stem cell factor, interleukin-1, interleukin-2, interleukin-3, interleukin-6, interleukin-7, interleukin-15, Flt3L, leukemia inhibitory factor, insulin-like growth factor, and insulin.
29. The method of Claim 5, wherein the at least one cytokine is stem cell factor, interleukin-7 and Flt3L, and wherein the at least one cytokine generates a cell population substantially enriched in colony-forming cells blast (CFC-Blast) stem cells.
30. The method of Claim 5, wherein the at least one cytokine is macrophage colony stimulating factor, interleukin-1, interleukin-3, interleukin-6 and stem cell factor, and wherein the at least one cytokine generates a cell population substantially enriched in hematopoietic high proliferative potential colony-forming cell (HPP-CFC) stem cells.
31. The method of Claim 5, wherein the at least one cytokine is erythropoietin, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, stem cell factor, interleukin-3, interleukin-6, and optionally Flt3L, and wherein the at least one cytokine generates a cell population substantially enriched in hematopoietic colony-forming cell erythroid, macrophage, megakaryocyte (CFC-GEMM) stem cells.
32. The method of Claim 5, wherein the at least one cytokine is selected from the group consisting of erythropoietin, erythropoietin and interleukin-3, erythropoietin and stem cell factor and erythropoietin, stem cell factor and

interleukin-3, and wherein the at least one cytokine generates a cell population substantially enriched in the hematopoietic burst forming unit-erythroid (BFU-E) progenitor cells.

- 5 33. The method of Claim 5, wherein the at least one cytokine is further selected from granulocyte-macrophage colony stimulating factor, granulocyte-macrophage colony stimulating factor and interleukin-3, and granulocyte-macrophage colony stimulating factor, interleukin-3 and stem cell factor, and wherein the at least one cytokine generates a cell population substantially
10 enriched in hematopoietic granulocyte-macrophage colony-forming cell (GM-CFC) progenitor cells.
34. The method of Claim 5, wherein the at least one cytokine is further selected from the groups consisting of thrombopoietin, and thrombopoietin,
15 interleukin-3 and interleukin-6, and wherein the at least one cytokine generates a cell population substantially enriched in the hematopoietic megakaryocyte colony-forming cell (CFC-Mega) progenitor cells.
35. The method of Claim 5, wherein the at least one cytokine is further selected from interleukin-2, and interleukin-7, Flt3L and interleukin-15, and wherein
20 the at least one cytokine generates a cell population substantially enriched in the hematopoietic T cell colony forming cell (T-CFC) progenitor cells.
36. The method of Claim 5, wherein the at least one cytokine is selected from the group consisting of interleukin-7, and interleukin-7 and Flt3L, and wherein the
25 at least one cytokine generates a cell population substantially enriched in the hematopoietic B cell colony-forming cell (B-CFC) progenitor cells.
37. The method of Claim 5, wherein the at least one cytokine is erythropoietin and
30 wherein the at least one cytokine generates a cell population substantially

enriched in the hematopoietic colony-forming unit-erythroid (CFU-E) progenitor cells.

38. The method of Claim 5, wherein the at least one cytokine is selected from the group consisting of granulocyte-colony stimulating factor and granulocyte-macrophage colony stimulating factor, and wherein the at least one cytokine generates a cell population substantially enriched in the hematopoietic granulocyte colony-forming cell (G-CFC) progenitor cells.

39. The method of Claim 5, wherein the at least one cytokine is selected from the group consisting of interleukin-3, and interleukin-3 and stem cell factor, and wherein the at least one cytokine generates a cell population substantially enriched in the hematopoietic colony-forming cell-Basophil (CFC-Bas) progenitor cells.

40. The method of Claim 5, wherein the at least one cytokine granulocyte-macrophage colony stimulating factor, interleukin-3 and interleukin-5, and wherein the at least one cytokine generates a cell population substantially enriched in the hematopoietic colony-forming cell-eosinophil (CFC-Eo) progenitor cells.

41. The method of Claim 5, wherein the at least one cytokine is selected from the group consisting of macrophage colony stimulating factor, macrophage colony stimulating factor and granulocyte-macrophage colony stimulating factor and interleukin-7, and granulocyte-macrophage colony stimulating factor, and wherein the at least one cytokine generates a cell population substantially enriched in the hematopoietic macrophage colony-forming cell (M-CFC) progenitor cells.

42. The method of Claim 1, further comprising the step of identifying a population

of primitive hematopoietic cells having a proliferative status suitable for transplantation into a recipient patient.

43. The method of Claim 1, wherein a population of primitive hematopoietic cells comprises a target cell population, and further comprising the steps of:
- (i) contacting the target cell population with a test compound; and
 - (ii) determining the ability of the test compound to modulate the proliferation, and optionally differentiation, of the target cell population.
44. The method of Claim 1, wherein the population of primitive hematopoietic cells comprises a plurality of target cell populations, and further comprising the steps of:
- (i) contacting the plurality of target cell populations with at least one test compound; and
 - (ii) determining the ability of the at least one test compound to alter the proliferation of the target cell population by comparing the proliferative status of the plurality of target cell populations with the proliferative status of a target population of primitive hematopoietic cells not in contact with the test compound; and
 - (iii) identifying the at least one test compound modulating the proliferative status of a target cell population.
45. A high-throughput assay method for rapidly identifying a population of primitive hematopoietic cells having a proliferative status suitable for transplantation into a patient, comprising the steps:
- (a) providing a cell population comprising primitive hematopoietic cells;
 - (b) incubating the cell population in a cell growth medium comprising a concentration of fetal bovine serum between 0%

and 30% and a concentration of methyl cellulose between about 0.4% and about 0.7%, and in an atmosphere having between about 3.5% oxygen and 7.5% oxygen;

- 5 (c) contacting the primitive hematopoietic cell population with at least one cytokine selected from the group consisting of erythropoietin, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, thrombopoietin, stem cell factor, interleukin-1, interleukin-2, interleukin-3, interleukin-6, 10 interleukin-7, interleukin-15, Flt3L, leukemia inhibitory factor, insulin-like growth factor, and insulin;
- (d) contacting the cell population with a reagent capable of generating luminescence in the presence of ATP; and
- 15 (e) detecting luminescence generated by the reagent contacting the at least two cell populations, the level of luminescence indicating the proliferative status of the primitive hematopoietic cells, and wherein the proliferative status of the primitive hematopoietic cells indicates the suitability of the cell population for transplantation into a recipient patient.
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46. The method of Claim 45, wherein contacting the population of primitive hematopoietic cells with at least one cytokine generates a cell population substantially enriched in a hematopoietic stem cell lineage.
- 25 47. The method of Claim 46, wherein the hematopoietic stem cell lineage is selected from the group consisting of colony-forming cell-blast (CFC-blast), high proliferative potential colony forming cell (HPP-CFC) colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM).
- 30 48. The method of Claim 45, wherein contacting the population of primitive

hematopoietic cells with at least one cytokine generates a cell population substantially enriched in at least one hematopoietic progenitor cell lineage.

49. The method of Claim 48, wherein the at least one hematopoietic progenitor cell lineage selected from the group consisting of granulocyte-macrophage colony-forming cell (GM-CFC), megakaryocyte colony-forming cell (CFC-mega), macrophage colony-forming cell (M-CFC), granulocyte colony forming cell (G-CFC), burst-forming unit erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), colony-forming cell-basophil (CFC-Bas), colony-forming cell-eosinophil (CFC-Eo), colony-forming cell-megakaryocyte (CFC-Mega), B cell colony-forming cell (B-CFC) and T cell colony-forming cell (T-CFC).
50. A high-throughput assay method for rapidly identifying a compound capable of modulating the proliferative status of a population of primitive hematopoietic cells, comprising the steps:
- (a) providing a first target cell population comprising primitive hematopoietic cells;
 - (b) incubating the cell population in a cell growth medium comprising a concentration of fetal bovine serum between 0% and 30% and a concentration of methyl cellulose between about 0.4% and about 0.7%, and in an atmosphere having between about 3.5% oxygen and 7.5% oxygen;
 - (c) providing a plurality of second target cell populations comprising primitive hematopoietic cells;
 - (d) contacting the first and second target primitive hematopoietic cell populations with at least one cytokine selected from the group consisting of erythropoietin, granulocyte-macrophage colony stimulating factor, granulocyte colony stimulating factor, macrophage colony stimulating factor, thrombopoietin,

stem cell factor, interleukin-1, interleukin-2, interleukin-3, interleukin-6, interleukin-7, interleukin-15, Flt3L, leukemia inhibitory factor, insulin-like growth factor, and insulin;

(e) contacting the first and second target cell populations with at least one test compound; and

(f) contacting the first and second target cell populations with a reagent capable of generating luminescence in the presence of ATP;

(g) detecting luminescence generated by the reagent contacting the first and second target cell populations, the level of luminescence indicating the proliferative status of the primitive hematopoietic cells; and

(h) comparing the proliferative status of the plurality of the second target cell populations with the proliferative status of the first target population of primitive hematopoietic cells not in contact with the test compound, thereby identifying a test compound capable of modulating the proliferative status of a target cell population.

51. The method of Claim 50, wherein contacting the first and second populations of primitive hematopoietic cells with at least one cytokine generates cell populations substantially enriched in hematopoietic stem cells.

52. The method of Claim 51, wherein the hematopoietic stem cells are selected from the group consisting of colony-forming cell-blast (CFC-blast), high proliferative potential colony forming cell (HPP-CFC) colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM).

53. The method of Claim 50, wherein contacting the first and second populations of primitive hematopoietic cells with at least one cytokine generates cell

populations substantially enriched in at least one hematopoietic progenitor cell lineage.

54. The method of Claim 53, wherein the at least one hematopoietic progenitor cell lineage selected from the group consisting of granulocyte-macrophage colony-forming cell (GM-CFC), megakaryocyte colony-forming cell (CFC-mega), macrophage colony-forming cell (M-CFC), granulocyte colony forming cell (G-CFC), burst-forming unit erythroid (BFU-E), colony-forming unit-erythroid (CFU-E), colony-forming cell-basophil (CFC-Bas), colony-forming cell-eosinophil (CFC-Eo), colony-forming cell-megakaryocyte (CFC-Mega), B cell colony-forming cell (B-CFC) and T cell colony-forming cell (T-CFC).
55. The method of Claim 50, further comprising the steps of:
- (a) contacting a target cell population with at least two concentrations of a test compound; and
 - (b) calculating the IC50 of the test compound.
56. The method of Claim 50, further comprising the step of calculating the IC90 of the test compound.